

We claim:

1. A method for cleaving single-stranded nucleic acid sequences at a desired location, the method comprising the steps of:

- 5 (i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and
- 10
- 15 (ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

20

25

2. A method for cleaving single-stranded nucleic acid sequences at a desired location, the method comprising the steps of:

30

- (i) contacting the nucleic acid with a partially double-stranded oligonucleotide,

the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site; and

(ii) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

3. In a method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a part of the diversity of the family, the improvement being characterized in that the displayed at least a part of peptide, polypeptide or protein is encoded at least in part by a nucleic acid that has been cleaved at a desired location by a method comprising the steps of:

- (i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and
- (ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;
- the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

4. In a method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a part of the diversity of the family, the improvement being characterized in that the displayed peptide, polypeptide or protein is encoded by a DNA sequence comprising a nucleic acid that has been cleaved at a desired location by

(i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site; and

(ii) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

5. A method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a part of the diversity of the family, the method comprising the steps of:

(i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;

(ii) rendering the nucleic acids single-  
5 stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

10 (a) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement  
15 in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

20 (b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain  
25 the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the  
30 chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature; and

(iv) displaying a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids on the surface of the genetic package and collectively displaying at  
5 least a portion of the diversity of the family.

6. A method for displaying a member of a diverse family of peptides, polypeptides or proteins on the surface of a genetic package and collectively displaying at least a portion of the diversity of the  
10 family, the method comprising the steps of:

(i) preparing a collection of nucleic acids that code, at least in part, for members of the diverse family;

(ii) rendering the nucleic acids single-  
15 stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a  
20 partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the  
25 double-stranded region of the oligonucleotide having a Type II-S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site; and

(b) cleaving the nucleic acid solely at  
30 the Type II-S cleavage site formed by the complementation of the nucleic acid and the

single-stranded region of the  
oligonucleotide;

the contacting and the cleaving steps being  
performed at a temperature sufficient to maintain  
5 the nucleic acid in substantially single-stranded  
form, the oligonucleotide being functionally  
complementary to the nucleic acid over a large  
enough region to allow the two strands to  
associate such that cleavage may occur at the  
10 chosen temperature and at the desired location,  
and the restriction being carried out using a  
cleavage endonuclease that is active at the chosen  
temperature; and

(iv) displaying a member of the family of  
15 peptides, polypeptides or proteins coded, at least in  
part, by the cleaved nucleic acids on the surface of  
the genetic package and collectively displaying at  
least a portion of the diversity of the family.

7. A library comprising a collection of  
20 genetic packages that display a member of a diverse  
family of peptides, polypeptides or proteins and  
collectively display at least a portion of the  
diversity of the family, the library being produced  
using the methods of claims 3, 4, 5 or 6.

25 8. A library comprising a collection of  
genetic packages that display a member of a diverse  
family of peptides, polypeptides or proteins and that  
collectively display at least a portion of the family,  
the displayed peptides, polypeptides or proteins being  
30 encoded by DNA sequences comprising at least in part  
sequences produced by cleaving single-stranded nucleic

acid sequences at a desired location by a method comprising the steps of:

- 5 (i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on  
10 restriction results in cleavage of the nucleic acid at the desired location; and
- 15 (ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the  
20 oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction  
25 endonuclease that is active at the chosen temperature.

9. A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and that collectively display at least a portion of the  
30 diversity of the family of the displayed peptides, polypeptides or proteins being encoded by DNA sequences comprising at least in part sequences produced by



cleaving single-stranded nucleic acid sequences at a desired location by a method comprising the steps of:

- 5 (i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a Type II S restriction endonuclease recognition site, whose cleavage site is located at a known distance from the recognition site where the cleavage of the nucleic acid is desired; and
- 10 (ii) cleaving the nucleic acid solely at the Type II-S cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;
- 15 the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the
- 20 two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

10. The methods according to any one of  
30 claims 1 to 9, wherein the nucleic acids encode at least a portion of an immunoglobulin.

11. The methods according to claim 10,  
wherein the immunoglobulin comprises a Fab or single  
chain Fv.

12. The methods according to claim 10 or 11,  
5 wherein the immunoglobulin comprises at least portion of  
a heavy chain.

13. The methods according to claim 12,  
wherein at least a portion of the heavy chain is human.

14. The methods according to claim 10 or 11,  
10 wherein the immunoglobulin comprises at least a portion  
of FR1.

15. The methods according to claim 14,  
wherein at least a portion of the FR1 is human.

16. The methods according to claim 10 or 11,  
15 wherein the immunoglobulin comprises at least a portion  
of a light chain.

17. The methods according to claim 16,  
wherein at least a portion of the light chain is human.

20 18. The methods according to any one of  
claims 1 to 9, wherein the nucleic acid sequences are  
at least in part derived from patients suffering from  
at least one autoimmune disease and/or cancer.

19. The methods according to claim 18,  
25 wherein the autoimmune disease is selected from the  
group comprising lupus, erythematosus, systemic

sclerosis, rheumatoid arthritis, antiphospholipid syndrome or vasculitis.

20. The methods according to claim 18,  
wherein the nucleic acids are at least in part isolated  
5 from the group comprising peripheral blood cells, bone  
marrow cells spleen cells or lymph node cells.

21. The methods according to claim 5 or 6  
further comprising an nucleic acid amplification step  
between steps (i) and (ii), between steps (ii) and  
10 (iii) or between steps (iii) and (iv).

22. The methods according to claim 21,  
wherein the amplification step uses geneRACE™.

23. The methods according to any one of  
claims 1 to 9, wherein the temperature is between 45°C  
15 and 75°C.

24. The methods according to claim 23,  
wherein the temperature is between 50°C and 60°C.

25. The methods according to claim 24,  
wherein the temperature is between 55°C and 60°C.

20 26. The methods according to claim 1, 3, 5  
or 8, wherein the length of the single-stranded  
oligonucleotide is between 17 and 30 bases.

27. The methods according to claim 26,  
wherein the length of the single-stranded  
25 oligonucleotide is between 18 and 24 bases.

28. The methods according to claim 1, 3, 5 or 8, wherein the restriction endonuclease is selected from the group comprising *MaeIII*, *Tsp45I*, *HphI*, *BsaJI*, *AluI*, *BlpI*, *DdeI*, *BglII*, *MslI*, *BsiEI*, *EaeI*, *EagI*,  
5 *HaeIII*, *Bst4CI*, *HpyCH4III*, *HinfI*, *MlyI*, *PleI*, *MnlI*, *HpyCH4V*, *BsmAI*, *BpmI*, *XmnI*, or *SacI*.

29. The methods according to claim 28, wherein the restriction endonuclease is selected from the group comprising *Bst4CI*, *TaaI*, *HpyCH4III*, *BlpI*,  
10 *HpyCH4V* or *MslI*.

30. The methods according to claim 2, 4, 6 or 9, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 14 and 22 bases.

15 31. The methods according to claim 30, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 14 and 17 bases.

32. The methods according to claim 31,  
20 wherein the length of the single-stranded region of the oligonucleotide is between 18 and 20 bases.

33. The methods according to claim 2, 4, 6 or 9, wherein the length of the double-stranded region of the partially double-stranded oligonucleotide is  
25 between 10 and 14 base pairs formed by a stem and its palindrome.

34. The methods according to claim 33 wherein, the partially double-stranded oligonucleotide comprises a loop of 3 to 8 bases between the stem and the palindrome.

5 35. The methods according to claim 2, 4, 6 or 9, wherein the Type II-S restriction endonuclease is selected from the group comprising AarICAC, AceIII, Bbr7I, BbvI, BbvII, Bce83I, BceAI, BceI, BciVI, BfiI, BinI, BscAI, BseRI, BsmFI, BspMI, EciI, Eco57I, FauI,  
10 FokI, GsuI, HgaI, HphI, MboII, MlyI, MneI, MnlI, PleI, RleAI, SfaNI, SspD5I, Sth132I, StsI, TaqII, Tth111II, or UbaPI.

36. The methods according to claim 35, wherein the Type II-S restriction endonuclease is FokI.

15 37. A method for preparing single-stranded nucleic acids for cloning into an vector, the method comprising the steps of:

(i) contacting a single-stranded nucleic acid sequence that has been cleaved with a  
20 restriction endonuclease with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region that remains after  
25 cleavage, the double-stranded region of the oligonucleotide including any sequences necessary to return the sequences that remain after cleavage into proper and original reading frame for expression and containing a  
30 restriction endonuclease recognition site 5' of those sequences; and

(ii) cleaving the partially double-stranded oligonucleotide sequence solely at the restriction endonuclease recognition site contained within the double-stranded region of the partially double-stranded oligonucleotide.

5

38. The method according to claim 37, wherein the length of the single-stranded portion of the partially double-stranded oligonucleotide is between 2 and 15 bases.

10

39. The method according to claim 38, wherein the length of the single-stranded portion of the partially double-stranded oligonucleotide is between 7 and 10 bases.

15

40. The method according to claim 37, wherein the length of the double-stranded portion of the partially double-stranded oligonucleotide is between 12 and 100 base pairs.

20

41. The method according to claim 40, wherein the length of the double-stranded portion of the partially double-stranded oligonucleotide is between 20 and 100 base pairs.